Characterizing the Inducible 1-Cysteine Peroxiredoxin Promoter in *Plasmodium berghei*

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ABSTRACT

Malaria is one of the world's deadliest infectious diseases, with over 200 million infections and hundreds of thousands of deaths annually. The malaria parasite *Plasmodium berghei* is a complex protozoan organism that requires two hosts: the human, site of asexual reproduction, and the *Anopheles* mosquito, where sexual reproduction occurs. My research project seeks to understand the antioxidant defense mechanisms *P. berghei* employs to withstand the hostile extracellular environment of the *Anopheles* mosquito vector through upregulation of 1-Cysteine Peroxiredoxin (1-CPrx). When activated, defense gene 1-CPrx counteracts reactive oxygen species (ROS) released by the mosquito as an immune response. Our goal is to determine the mechanisms that allow the parasite to sense an environmental change and respond through upregulation of 1-CPrx. We hypothesize the activation of 1-CPrx promoter is driven by an *antioxidant response element* (ARE), which upregulates the defense gene.

BACKGROUND

To effectively bridge the gap from the intermediate human host to the mosquito, *Plasmodium* must re-organize its cell biology to adapt to the unique cellular conditions in each host. Migration through the midgut of the mosquito is a dangerous feat for *Plasmodium*, openly exposed to the mosquito's defense mechanisms; immersed in the mosquito's blood meal, *Plasmodium* faces threat of digestion and risk of damage by cytotoxic reactive oxygen (ROS) and reactive nitrogen species (RNS). To counteract the mosquito's immune response, *Plasmodium* upregulates specific antioxidant defense genes (Fig. 1). The Kanzok lab determined that one of these antioxidant genes, 1-Cysteine Peroxiredoxin (1-CPrx), is strongly upregulated by the parasite to combat exposure to ROS in the mosquito midgut (Turturice et al 2013). Characterization of antioxidant defense genes is of importance to us because they serve as attractive targets for the development of transmission blocking strategies to combat malaria. My **objective** is to determine the location of the ARE in the promoter of 1-CPrx using a "promoter-bashing" approach, established by former lab student Cecile Swift. I will utilize a series of luciferase reporter constructs and transfect them into *P. berghei* to study the 1-CPrx promoter. Using this method, I will map the promoter of 1-CPrx to find the location of the ARE and characterize the first inducible promoter of the malaria parasite *Plasmodium* (Fig. 2).

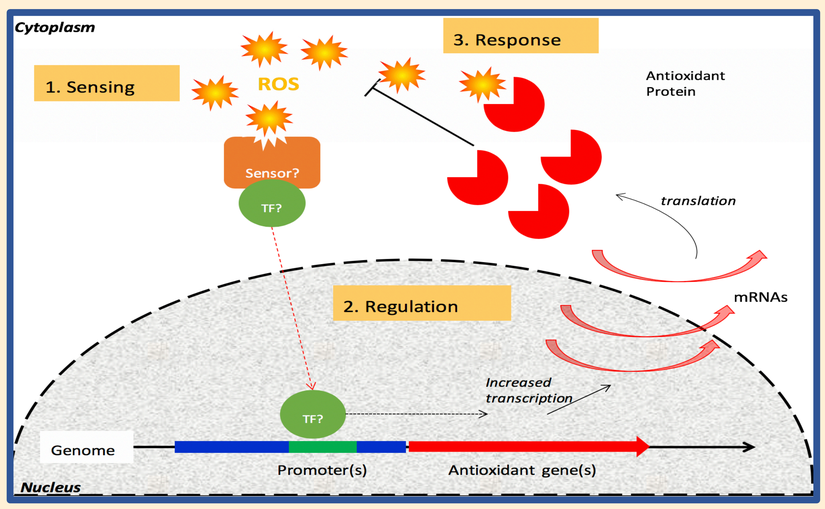


Figure 1: *Plasmodium*'s Regulation of Oxidative Stress Response via 1-CPrx Upregulation (Swift et al., in prep)

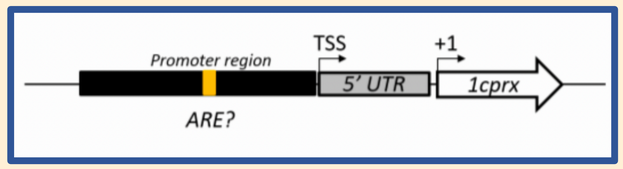
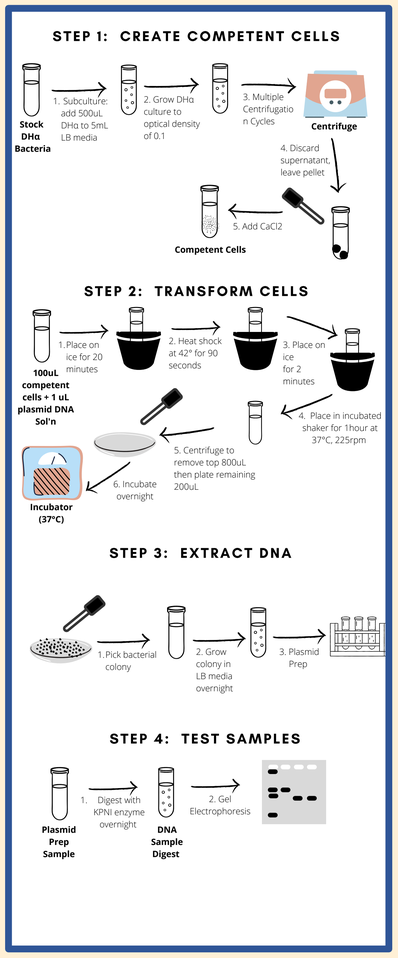


Figure 2: Promoter contains *Antioxidant Response Elements* (Swift et al., in prep)

METHODS

Using the transfection protocol, pilot data for 2.4kb and 1.0kb 1-CPrx promoter fragments with comparable luciferase signals were generated by Swift. Luminescence of the 1.0kb fragment indicates the presence of the minimal promoter region. From Swift's pilot data, I have generated numerous plasmids containing the 0.5kb and 1.0kb 1-CPrx promoter fragments through bacterial transformation to begin the process of locating the ARE within the 1.0kb fragment. To ensure the presence of the 1-CPrx gene of interest, DNA samples were then subjected to restriction digests using restriction enzyme KPNI, followed by analytical gel electrophoresis of the digested sample to confirm the bacteria had been successfully transformed with the 1-CPrx promoter fragments (Fig. 3).

Figure 3: Transformation of Bacteria and DNA Extraction



RESULTS

To generate results for my research project, I must create my "molecular toolbox," which involves (1) generation of excess insert DNA (K fragments, removed from plain PDLC vector); (2) generation of vector DNA—PDLC 0.5ΔK and PDLC 1.0 ΔK; (3) practice handling, infecting, and exsanguinating mice to be transfected with target DNA.

ONGOING PROJECT

Using the purified 1-CPrx promoter fragments, luciferase reporter plasmids will be generated, followed by transfection of *P. berghei* with reporter plasmids and subsequent quantification of the 1-CPrx promoter in the luciferase assay. The reporter plasmid is transfected into the parasite via electroporation, then the mouse is infected. Activation of 1-CPrx leads to the subsequent activation of the luc gene, generating luminescence; luminescence is quantified using a luminometer relative to a control group (a *Renilla* luc protein coupled to a control promoter). A truncated 1-CPrx gene containing the promoter should yield a strong luminescence signal. Currently, I have completed mouse training in the ACF and am learning how to infect mice with parasites via intraperitoneal and tail-vein injections, perform tail snips for blood smears, determine parasitemia, and exsanguinate infected mice to obtain parasites. Once I have gained more experience handling mice, I will transfect mice with my luciferase reporter constructs. I will also use computational methods to analyze the 1-CPrx promoter for potential transcription factor binding sites. The goal is to determine the location of the ARE by generating systematically smaller fragments and analyze resulting luminescence. A fragment exhibiting no luminescence implies the inducible region is no longer present. Once identified, I will screen the *Plasmodium* genome for additional ARE sites on other genes.

DISCUSSION

My long-term goals will encompass a) removal of the putative TF-binding site from the parasite genome to determine whether 1-CPrx expression is negatively affected and results in increased sensitivity to ROS in the malaria parasite, b) using the TF-binding site as bait to identify the transcription factor that binds to the TF-binding site and regulates 1-CPrx expression, c) using computational methods to screen the *Plasmodium* genome for presence of newly identified TF-binding site(s) and TFs. Localization of the ARE could be used to determine the transcription factor that leads to its activation and will provide new insight into the mechanism of antioxidant response in *Plasmodium*.

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